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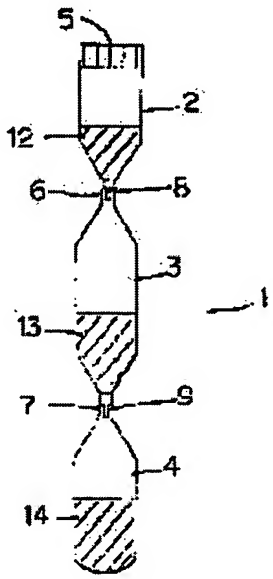
(54) METHOD AND KIT FOR DETECTING ENDOTOXINE IN STOCK BLOOD

(57)Abstract:
PURPOSE: To obtain kit and method for detecting endotoxine in stock blood for transfusion in order to prevent transfusion of blood contaminated with bacteria.
CONSTITUTION: The method for detecting endotoxine in stock blood comprises a step for causing a sample to react with a main agent 12 in a reaction bath 2, a step for bringing the sample and the main agent 12 into contact with a reaction stop agent 13 in a reaction stop bath 3, and an optional step for bringing the sample into contact with a coloring agent 14 in a coloring bath 4. Since contamination of transfusion blood with bacteria can be determined not by visual inspection (subjective) but by colorimetric inspection (objective), the inventive method contributes greatly to prevention of accident of transfusion.



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CLAIMS

[Claim(s)]
[Claim 1] The endotoxin detection approach in the stored blood characterized by consisting of each following process.
(1) The process to which a sample is made to react with base resin in a reaction vessel, the process which contacts a reaction halt agent to said sample and base resin in (2) reaction halt tub or the process which contacts a coloring agent in said sample in (3) coloring tubs following the process of the above (2), [Claim 2] The endotoxin detection approach in the stored blood characterized by consisting of each following process.
(1) the process which passes an adsorbent for a sample and makes endotoxin stick to an adsorbent, and (2) -- the process to which said endotoxin and base resin are made to react, and (3) -- the process which contacts a reaction halt agent to said endotoxin and base resin, or the process of the above (3) -- continuing -- (4) -- the process and [Claim 3] which contact a coloring agent to said endotoxin A reaction vessel and a reaction halt tub are connected through the communication trunk which has arranged the free passage member on the way. Or a reaction halt tub and a coloring tub are connected through the communication trunk which has arranged the free passage member on the way. The endotoxin detection kit characterized by what base resin was enclosed with the interior while the upper part of said reaction vessel was equipped with the plug, the reaction halt agent was enclosed with the interior of said reaction halt tub, or the coloring agent was enclosed with the interior of said coloring tub for.
[Claim 4] The endotoxin detection kit characterized by what the specific adsorbent of endotoxin and the preservative for maintaining the activity of this adsorbent were enclosed with the interior, the upper part was equipped with the plug, and waste fluid opening was formed in the lower part for.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the detection kit and its detection approach of endotoxin in the stored blood for blood transfusion for preventing beforehand blood transfusion of blood by which contamination was carried out.
[0002]
[Description of the Prior Art] The endotoxin quanta in plasma have the Wako Pure Chem kit by the gelling method Limulus test, and the Seikagaku kit by the coloring method using a coloring composition substrate as drugs for an external diagnosis. For example, an example of operating procedure which detects endotoxin, using the drugs for an external diagnosis (endotoxin quantum reagent in plasma "endotoxin test-D") as a Seikagaku kit is as follows.
[0003] (1) An aluminium cap is made an endotoxin free test tube, carry out 150gx10min centrifugal [of the 1ml of the 4 degrees C of the blood of specimen extraction and preparation heparin blood collecting], obtain PRP (platelet rich plasma), and save under ice-cooling.
(2) -1 Pretreatment (deproteinization processing)
PRP (200microl) of (1) -- a perchloric acid solution (400microl) -- adding -- a mixer -- after churning, and 37 degrees C and 20min -- warming -- it carries out after 1000gx15min centrifugal.
(2) -2 100micro of sodium-hydroxide solutions l is added to 100micro of neutralization (2) supernatant liquid l of -1, and it neutralizes.
(3) Add 100micro of (2) specimens l of -2 to 160micro (limulus-amebocyte-lysate + coloring composition substrate + buffer solution) of main reaction base resin l, and agitate by the mixer. (37 degrees C is warmed 30 min)
(4) Reaction halt (endotoxin free actuation need)
A nitrous acid Na and 500micro of solution of hydrochloric acid l are promptly added after ice-cooling, and it agitates well.
(5) Add and agitate 500micro of ammonium-sulfamate solutions l for a diazo coupling reaction (4), and, subsequently add and agitate 500micro of N-(1-naphthyl) ethylenediamine dihydrochloride l.
(6) Measure an absorbance for measurement red with a spectrophotometer on the wavelength of 545nm.
Caution sufficient [all have complicated actuation, and / when taking time amount, actuation of an endotoxin free-lancer besides an experimenter's mastery is required, and] in order to obtain a reliable result is required.
[0004] The retention period of the blood product transfused on the other hand became possible for a long period of time by development of an anticoagulant and erythrocyte preservation liquid, and the refrigeration preservation of an erythrocyte preservation liquid (MAP liquid) addition erythrocyte strong solution will be attained also at home on a maximum of the 42nd. Therefore, with mothball-izing, the contamination problem by cold growing bacteria, such as the Yersinia bacillus, arises, the example which died by blood transfusion of contamination blood overseas is also reported, and the report of the Yersinia bacillus detection is also made from the inside of stored blood at home. Moreover, the report of

contamination by growth of serratia marcescens etc. also has the platelet pharmaceutical preparation saved at 22 degrees C except erythrocyte pharmaceutical preparation. Although it has been judged by a remarkable hemolysis and the existence of discoloration and aggregate formation by the appearance-opinion before blood transfusion whether the blood for blood transfusion is polluted by bacteria until now, it is thought difficult for the extent to except thoroughly [change with the strain of the cause of contamination, classes of blood product, etc. in many cases, and / in visual inspection]. Then, this invention person came to invent the detection approach of the endotoxin for preventing blood transfusion of contamination blood beforehand, and the detection kit by measuring the endotoxin in blood simple and promptly, when the above blood for blood transfusion is polluted by bacteria, as a result of repeating examination wholeheartedly.

[0005]

[Means for Solving the Problem]

[1] This invention offers the endotoxin detection approach in the stored blood which consists of each following process.

(1) The process to which a sample is made to react with base resin in a reaction vessel, the process which contacts a reaction halt agent to said sample and base resin in (2) reaction halt tub or the process which contacts a coloring agent in said sample in (3) coloring tubs following the process of the above (2), and [2] this inventions offer the endotoxin detection approach in the stored blood which consists of each following process.

(1) The process which passes an adsorbent for a sample and makes endotoxin stick to an adsorbent, (2) -- the process to which said endotoxin and base resin are made to react, and (3) -- the process which contacts a reaction halt agent to said endotoxin and base resin -- The process of the above (3) is followed. Or the process which contacts a coloring agent to said endotoxin, and (4) [3] this invention A reaction vessel and a reaction halt tub are connected through the communication trunk which has arranged the free passage member on the way. Or a reaction halt tub and a coloring tub are connected through the communication trunk which has arranged the free passage member on the way. While the upper part of said reaction vessel is equipped with a plug, base resin is enclosed with the interior, a reaction halt agent is enclosed with the interior of said reaction halt tub, and the interior of said coloring tub is provided with the endotoxin detection kit which enclosed the coloring agent.

[4] The specific adsorbent of endotoxin and the preservative for maintaining the activity of this adsorbent are enclosed with the interior, the upper part is equipped with a plug, and this invention offers the endotoxin detection kit which formed waste fluid opening in the lower part.

[0006]

[Example] Drawing 1 is the schematic diagram of the endotoxin detection kit 1 (henceforth, detection kit 1), the detection kit 1 consists of tubed reaction vessels 2, reaction halt tubs 3, and coloring tubs 4, a reaction vessel 2 and the reaction halt tub 3 are connected through the communication trunk 6 which has arranged the free passage member 8 on the way, and the reaction halt tub 3 and the coloring tub 4 are connected through the communication trunk 7 which has arranged the free passage member 9 on the way. The upper part of a reaction vessel 2 is equipped with a plug 5, base resin 12 is enclosed with the interior of a reaction vessel 2, the reaction halt agent 13 is enclosed with the interior of the reaction halt tub 3, and the coloring agent 14 is enclosed with the interior of the coloring tub 4. Each reaction vessel 2 and the reaction halt tub 3 which constitute the detection kit 1, and the coloring tub 4 are maintained by the closed system. Reaction-vessel 2 grade is pressed from the outside, and it is formed with a flexible plastic tube etc. so that a pumping can be carried out, and it is assembled by connecting by said communication trunk 6 grade, respectively. Moreover, although you may load with the free passage member 8 which becomes the interior of a communication trunk 6 (7) from the member of a major diameter and a minor diameter through a thin-walled part like drawing 1 as a means to arrange the free passage member 8 (9) to a communication trunk 6 (7), you may equip with the free passage member which becomes the periphery of a communication trunk 6 (7) from a clamp etc.

[0007] At this invention, they are limulus amebocyte lysate (rye SETO), a coloring composition substrate (Boc-Leu-Gly-Arg-pNA; t-butoxycarbonyl - L-leucyl-glycyl-L-arginine-p-nitroanilide

hydrochloride), and the buffer solution (for example) as said base resin 12. The constituent which consists of a phosphate buffer solution, the tris hydrochloric-acid buffer solution, the good buffer solution, etc. It is used. ((A) The following, base resin) etc. -- a sodium-nitrite-hydrochloric-acid water solution as said reaction halt agent 13 ((A) Following and reaction halt agent) etc. -- it is used and an ammonium-sulfamate solution, the mixture (the following, a coloring agent A) of N-(1-naphthyl) ethylenediamine dihydrochloride, etc. are used as said coloring agent 14.

[0008] Drawing 2 is the schematic diagram of the other endotoxin detection kits 21 (henceforth, detection kit 21), the preservative 23 for the detection kit 21 to maintain the activity of the specific adsorbent 22 (the following, adsorbent 22) of endotoxin and this adsorbent 22 inside is enclosed, the upper part is equipped with a plug 25, and the waste fluid opening 29 is formed in the lower part. The lower part of the waste fluid opening 29 is sealed with means, such as joining, at the time of an activity, it may be made to carry out cutting opening of it with scissors etc., and it equips with the plug, and it may form it so that opening of the plug may be removed and carried out at the time of an activity. In this invention, the PAIRO sep A by Daicel Chemical Industries, Ltd. (the software gel type, adsorbent (A) which carried out covalent bond of the histidine to agarose by making a hexamethylenediamine into a spacer), the PAIRO sep C (the hard gel type, adsorbent (C) which used the cellulose as the base), etc. are used as said adsorbent 22, and water (preservative A), the low ionic strength buffer solution (preservative B), a sodium chloride water solution (for example, 0.05M) (preservative C), etc. are used as a preservative 23. Moreover, deproteinization processing of the pharmaceutical preparation which the pharmaceutical preparation (whole blood, erythrocyte strong solution) which contains an erythrocyte in this invention, and the pharmaceutical preparation (plasma, ischemia scutellum plasma, platelet rich plasma, platelet strong solution) which does not contain an erythrocyte are used, the supernatant is used after carrying out centrifugal processing of the pharmaceutical preparation containing an erythrocyte, and does not contain an erythrocyte is carried out, and the supernatant is used after carrying out centrifugal processing. Moreover, in this invention, the color of coloring can be freely set up by choosing a coloring agent.

[0009] Next, the example of detection of endotoxin is explained.

Centrifugal processing of the platelet rich plasma which carried out example 1 deproteinization processing was carried out (when the detection kit 1 detects), these supernatants were extracted in the test tube, and Sample A was created. Sample A was extracted to the syringe, and the puncture of this was carried out to the plug 5, and it poured in into the reaction vessel 2, and was made to react for 20 minutes at base resin A (for the buffer solution to have used the phosphate buffer solution.) and a room temperature. The free passage member 8 was fractured, said Sample A and base resin A were introduced into the reaction halt tub 3, and the reaction halt agent A was contacted. Fracture the free passage member 8, introduce said sample A etc. into the coloring tub 4, the coloring agent A was made to contact, the diazo coupling reaction was produced, and the coloring situation in the coloring tub 4 was observed with the naked eye. It was able to be faintly colored red and, thereby, the inside of the coloring tub 4 was able to detect endotoxin. By the approach of this example, all actuation until it results [from pretreatment of Sample A] in coloring observation of the red of the coloring tub 4 was able to be performed in 50 minutes (coloring 30 minutes from pretreatment 20 minutes, and a reaction).

[0010] The sample A created like example 2 example 1 was extracted to the syringe (when the detection kit 21 detects), and the puncture of this was carried out to the plug 25, and it poured in into the detection kit 21, and was left at the room temperature for 30 minutes. Endotoxin discharged other Samples A and Preservatives A from the waste fluid opening 29, after Adsorbent C was adsorbed. Base resin A was added into the detection kit 21, and it was made to react for 20 minutes with said endotoxin. Then, the reaction halt agent A and the coloring agent A were added to these one by one, the diazo coupling reaction was produced, and the coloring situation in the detection kit 1 was observed with the naked eye. It was able to be faintly colored red and, thereby, the inside of the detection kit 1 was able to detect endotoxin. By the approach of this example, all actuation until it results [from pretreatment of Sample A] in coloring observation of the detection kit 25 was able to be performed in 60 minutes.

[0011] After setting the reaction temperature of Sample A and base resin A as the elevated temperature

(37 degrees C) in the example 3 aforementioned example 1 and the example 2 and promoting a reaction, the reaction halt agent A was contacted and the coloring situation in the reaction halt tub 4 (detection kit 21) was observed with the naked eye. It was able to be faintly colored yellow and, thereby, the inside of the reaction halt tub 4 (detection kit 21) was able to detect endotoxin. Moreover, the amount of a sample was increased, and even if it makes concentration of base resin A etc. high and made Sample A and base resin A react at a room temperature, endotoxin was detectable like the above. At this example, all actuation was able to be performed in 40 minutes until it resulted [from pretreatment of Sample A] in coloring observation of endotoxin.

[0012] Moreover, when detecting endotoxin like an example 3 in this invention, in the detection kit 1 of drawing 1 , a communication trunk 9 and the coloring tub 4 can be omitted.

[0013] In this invention, the detection sensitivity of endotoxin is changeable by adjusting suitably the amount of the amount of the sample used for a trial, reaction time, temperature, base resin, and a coloring agent. Moreover, if the sample of the red corresponding to the amount of endotoxins which should be recognized is prepared when a blood product pollutes, it will examine with this kit and the judgment of contamination blood will become easy by comparing a red coloring condition.

[0014]

[Function and Effect of the Invention]

(1) By changing the check of the contamination of the blood for blood transfusion into colorimetry (objective) inspection from the appearance (subjective) inspection by old viewing, it can contribute to a blood-transfusion avoid accident greatly.

(2) Even if preparation of an instrument and a reagent does not take time amount and it is not under a clean bench compared with the conventional endotoxin detecting method (for an external diagnosis), detection of endotoxin can be performed for a short time. (all the processes of detection actuation -- conventional method: -- about 1.5 - 2 hours -- receiving -- this invention:40 - 60 minutes)

(3) As front [blood transfusion] inspection, blood transfusion of contamination blood can be beforehand prevented by using this kit.

(4) In the conventional method, in order to begin the test tube pipet used since actuation of an endotoxin free-lancer is required and to prevent contamination of the endotoxin from other than a specimen from specimen adjustment to a reaction halt phase, it needed to examine under pure environments, such as a clean bench, but by the approach of this invention, after extracting the blood product to measure in sterile by a DISUPO syringe etc., since it becomes a reaction within the kit of a closed system, it can carry out under the usual environment.

(5) Although reading and an exact quantum are possible for the conventional drugs for an external diagnosis in red at an absorbance, since the approach of this invention has set whether the blood for blood transfusion is polluted by bacteria in the 1st object to detect, it can be judged by judging red with a naked eye.

[Translation done.]

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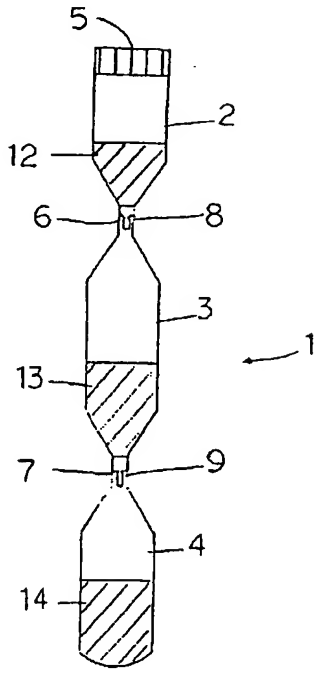
(54)【発明の名称】　保存血液中のエンドトキシン検出方法及び検出キット

(57)【要約】

【目的】　細菌汚染された血液の輸血を未然に防止するための輸血用保存血液中のエンドトキシンの検出キット及びその検出方法に関する。

【構成】　次の各工程よりなる保存血液中のエンドトキシン検出方法及び検出キット。（１）反応槽の中で試料を主剤と反応させる工程、（２）反応停止槽の中で前記試料と主剤に反応停止剤を接触させる工程、または前記（２）の工程に続いて（３）着色槽の中で前記試料に着色剤を接触させる工程、

【効果】　輸血用血液の細菌汚染の確認をこれまでの目視による外観（主観的）検査から比色（客観的）検査に変更することで、輸血事故防止に大きく貢献できる。



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【特許請求の範囲】

【請求項1】　次の各工程よりなることを特徴とする保存血液中のエンドトキシン検出方法。

（1）反応槽の中で試料を主剤と反応させる工程、

（2）反応停止槽の中で前記試料と主剤に反応停止剤を接触させる工程、または前記（2）の工程に続いて

（3）着色槽の中で前記試料に着色剤を接触させる工程、

【請求項2】　次の各工程よりなることを特徴とする保存血液中のエンドトキシン検出方法。

（1）試料を吸着剤を通過させて、エンドトキシンを吸着剤に吸着させる工程、（2）前記エンドトキシンと主剤を反応させる工程、（3）前記エンドトキシンと主剤に反応停止剤を接触させる工程、または前記（3）の工程に続いて（4）前記エンドトキシンに着色剤を接触させる工程、

【請求項3】　反応槽と反応停止槽を途中に連通部材を配置した接続管を介して接続し、または反応停止槽と着色槽を途中に連通部材を配置した接続管を介して接続し、

前記反応槽の上部には栓体が装着されるとともに内部には主剤が封入され、前記反応停止槽の内部には反応停止剤が封入され、または前記着色槽の内部には着色剤を封入した、ことを特徴とするエンドトキシン検出キット。

【請求項4】　内部にエンドトキシンの特異的吸着剤と、該吸着剤の活性を維持するための保存剤が封入され、

上部に栓体が装着され、下部に廃液口を形成した、ことを特徴とするエンドトキシン検出キット。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、細菌汚染された血液の輸血を未然に防止するための輸血用保存血液中のエンドトキシンの検出キット及びその検出方法に関するものである。

【0002】

【従来技術及び発明が解決しようとする課題】血漿中のエンドトキシン定量用に体外診断用医薬品として、ゲル化法リムルステストによる和光純薬製キット及び発色合成基質を用いた発色法による生化学工業製キットがある。例えば生化学工業製キットとして体外診断用医薬品（血漿中エンドトキシン定量試薬「エンドトキシンテストーD」）を用いてエンドトキシンの検出を行う操作手順の一例は以下の通りである。

【0003】（1）　検体採取と調製

ヘパリン採血の血液1mlをエンドトキシンフリー試験管にアルミキャップをし、4℃、150g×10min遠心しPRP（多血小板血漿）を得て、氷冷下で保存する。

（2）－1　前処理（除タンパク処理）

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（1）のPRP（200μl）を過塩素酸溶液（400μl）に加え、ミキサーで撹拌後、37℃、20min加温後1000g×15min遠心する。

（2）－2　中和

（2）－1の上清100μlに水酸化ナトリウム溶液100μlを加え、中和する。

（3）　主反応

主剤（カブトガニ血球抽出成分＋発色合成基質＋緩衝液）160μlに（2）－2の検体100μlを加え、ミキサーで撹拌する。（37℃、30min加温）

（4）　反応停止（エンドトキシンフリーの操作必要）

直ちに氷冷後、亜硝酸Na、塩酸溶液500μlを加え、よく撹拌する。

（5）ジアゾカップリング反応

（4）にスルファミン酸アンモニウム溶液500μlを加え撹拌し、次いでN－（1－ナフチル）エチレンジアミン二塩酸塩500μlを加え、撹拌する。

（6）　測定

赤色を波長545nmで分光光度計により吸光度を測定する。いずれも操作が繁雑で時間がかかる上、実験者の習熟の他、エンドトキシンフリーの操作が必要であり、信頼性のある結果を得るためには、十分な注意が必要である。

【0004】一方輸血される血液製剤は、抗凝固剤、赤血球保存液の開発により、保存期間は長期間可能となり、国内でも赤血球保存液（MAP液）添加赤血球濃厚液は最長42日まで冷蔵保存が可能となった。そのため長期保存化に伴い、エルシニア菌などの低温増殖菌による汚染問題が起こり、海外では汚染血液の輸血により死亡した例も報告され、国内においても保存血液中よりエルシニア菌検出の報告もなされている。また赤血球製剤以外でも22℃で保存される血小板製剤でもセラチア菌などの増殖による汚染の報告もある。これまで、輸血用血液が細菌に汚染されているかどうかは、輸血前の外観的所見により著しい溶血や変色、凝集塊形成の有無により判断されてきたが、その程度は汚染原因の菌種、血液製剤の種類などにより異なることが多く外観検査のみで完全に除外することは難しいと考えられる。そこで本発明者は、鋭意検討を重ねた結果上記のような輸血用血液が細菌に汚染されている場合に、血液中のエンドトキシンを簡便、迅速に測定することにより、汚染血液の輸血を未然に防止するためのエンドトキシンの検出方法及び検出キットを発明するに至った。

【0005】

【課題を解決するための手段】

［1］本発明は、次の各工程よりなる保存血液中のエンドトキシン検出方法を提供する。

（1）反応槽の中で試料を主剤と反応させる工程、

（2）反応停止槽の中で前記試料と主剤に反応停止剤を接触させる工程、または前記（2）の工程に続いて

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（3）着色槽の中で前記試料に着色剤を接触させる工程、

［2］本発明は、次の各工程よりなる保存血液中のエンドトキシン検出方法を提供する。

（1）試料を吸着剤を通過させて、エンドトキシンを吸着剤に吸着させる工程、（2）前記エンドトキシンと主剤を反応させる工程、（3）前記エンドトキシンと主剤に反応停止剤を接触させる工程、または前記（3）の工程に続いて（4）前記エンドトキシンに着色剤を接触させる工程、

［3］本発明は、反応槽と反応停止槽を途中に連通部材を配置した接続管を介して接続し、または反応停止槽と着色槽を途中に連通部材を配置した接続管を介して接続し、前記反応槽の上部には栓体が装着されるとともに内部には主剤が封入され、前記反応停止槽の内部には反応停止剤が封入され、前記着色槽の内部には着色剤を封入した、エンドトキシン検出キットを提供する。

［4］本発明は、内部にエンドトキシンの特異的吸着剤と、該吸着剤の活性を維持するための保存剤が封入され、上部に栓体が装着され、下部に廃液口を形成した、エンドトキシン検出キットを提供する。

【0006】

【実施例】図1はエンドトキシン検出キット1（以下、検出キット1）の概略図で、検出キット1は筒状の反応槽2と反応停止槽3と着色槽4より構成され、反応槽2と反応停止槽3は途中に連通部材8を配置した接続管6を介して接続され、反応停止槽3と着色槽4は途中に連通部材9を配置した接続管7を介して接続されている。反応槽2の上部には栓体5が装着され、反応槽2の内部には主剤12が封入され、反応停止槽3の内部には反応停止剤13が封入され、着色槽4の内部には着色剤14が封入されている。検出キット1を構成する各反応槽2と反応停止槽3と着色槽4は閉鎖系に維持されている。反応槽2等は外部から押圧してポンピングできるように可とう性のプラスチックチューブ等により形成され、それぞれ前記接続管6等により接続することにより組み立てられる。また接続管6（7）へ連通部材8（9）を配置する手段として図1のように接続管6（7）内部に薄肉部を介して大径と小径の部材よりなる連通部材8を装填しても良いが接続管6（7）の外周にクランプ等からなる連通部材を装着しても良い。

【0007】本発明では、前記主剤12としてカブトガニ血球抽出成分（ライセート）と発色合成基質（Boc－Leu－Gly－Arg－pNA；t－ブトキシカルボニル－L－ロイシル－グリシル－L－アルギニン－p－ニトロアニリド塩酸塩）と緩衝液（例えば、リン酸緩衝液、トリス塩酸緩衝液、グッド緩衝液等）からなる組成物（以下、主剤A）等が使用され、前記反応停止剤13として亜硝酸ナトリウム－塩酸水溶液（以下、反応停止剤A）等が使用され、前記着色剤14としてスルファミン酸アンモニウム溶液とN－（1－ナフチル）エチレンジアミン二塩酸塩の混合物（以下、着色剤A）等が使用される。

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【0008】図2はその他のエンドトキシン検出キット21（以下、検出キット21）の概略図で、検出キット21は内部にエンドトキシンの特異的吸着剤22（以下、吸着剤22）と該吸着剤22の活性を維持するための保存剤23が封入され、上部には栓体25が装着され、下部には廃液口29が形成されている。廃液口29の下部は、溶着等の手段により密封しておいて、使用時にハサミ等で切断開口するようにしても良いし栓体を装着しておいて、使用時に栓体をはずして開口するように形成しても良い。本発明では前記吸着剤22としてダイセル化学工業株式会社製のバイロセップA（アガロースにヘキサメチレンジアミンをスペーサーとして、ヒスチジンを共有結合させたソフトゲルタイプ、吸着剤A）、バイロセップC（セルロースをベースとしたハードゲルタイプ、吸着剤C）等が使用され、保存剤23として水（保存剤A）、低イオン強度緩衝液（保存剤B）、塩化ナトリウム水溶液（例えば0．05M）（保存剤C）等が使用される。また、本発明では赤血球を含む製剤（全血、赤血球濃厚液）と赤血球を含まない製剤（血漿、貧血小板血漿、多血小板血漿、血小板濃厚液）が使用され、赤血球を含む製剤は遠心処理した後、その上澄が使用され、赤血球を含まない製剤は、除蛋白処理して遠心処理した後、その上澄が使用される。また本発明では着色剤を選択することにより着色の色を自由に設定することができる。

【0009】次にエンドトキシンの検出例について説明する。

実施例1（検出キット1により検出を行う場合）

除蛋白処理した多血小板血漿を遠心処理し、これらの上澄を試験管に採取して試料Aを作成した。試料Aをシリンジに採取し、これを栓体5に穿刺して反応槽2の中に注入し、主剤A（緩衝液はリン酸緩衝液を使用した。）と室温で20分間反応させた。連通部材8を破断して前記試料Aと主剤Aを反応停止槽3の中に導入して、反応停止剤Aを接触させた。連通部材8を破断して前記試料A等を着色槽4の中に導入して、着色剤Aと接触させ、ジアゾカップリング反応を生じさせ着色槽4中の着色状況を肉眼で観察した。着色槽4内はかすかに赤色に着色し、これによりエンドトキシンを検出することができた。本実施例の方法では、試料Aの前処理から着色槽4の赤色の着色観察に至るまでの全操作を50分（前処理20分、反応から着色30分）で行うことができた。

【0010】実施例2（検出キット21により検出を行う場合）

実施例1と同様に作成した試料Aをシリンジに採取し、これを栓体25に穿刺して検出キット21の中に注入し、室温で30分放置した。エンドトキシンが吸着剤C

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に吸着された後、その他の試料Ａ、保存剤Ａは廃液口２９より廃出した。検出キット２１の中に主剤Ａを加え前記エンドトキシンと２０分間反応させた。続いてこれらに反応停止剤Ａ、着色剤Ａを順次加えてジアゾカップリング反応を生じさせ検出キット１中の着色状況を肉眼で観察した。検出キット１内はかすかに赤色に着色し、これによりエンドトキシンを検出することができた。本実施例の方法では、試料Ａの前処理から検出キット２５の着色観察に至るまでの全操作を６０分で行うことができた。

【００１１】実施例３

前記実施例１及び実施例２において試料Ａと主剤Ａの反応温度を高温（３７℃）に設定して反応を促進させた後、反応停止剤Ａを接触させて反応停止槽４（検出キット２１）内の着色状況を肉眼で観察した。反応停止槽４（検出キット２１）内はかすかに黄色に着色し、これによりエンドトキシンを検出することができた。また、試料の量を増やし、主剤Ａ等の濃度を高くして室温で試料Ａと主剤Ａを反応させても前記と同様にエンドトキシンを検出することができた。本実施例では試料Ａの前処理からエンドトキシンの着色観察に至るまで全操作を４０分で行うことができた。

【００１２】また、本発明において実施例３のようにエンドトキシンの検出を行う場合は、図１の検出キット１において接続管９と着色槽４は省略することができる。

【００１３】本発明においては、試験に使用する試料の量、反応時間、温度、主剤、着色剤の量を適宜調整することにより、エンドトキシンの検出感度を変えることができる。また、血液製剤が汚染した場合、認知すべきエンドトキシン量に見合った赤色の見本を用意しておけば、本キットにより試験を行い、赤色の着色状態を比べることで、汚染血液の判定は容易となる。

【００１４】

【発明の作用効果】

（１）輸血用血液の細菌汚染の確認をこれまでの目視による外観（主観的）検査から比色（客観的）検査に変更することで、輸血事故防止に大きく貢献できる。

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（２）従来のエンドトキシン検出法（体外診断用）に比べ、器具、試薬の準備に時間がかからずクリーンベンチ下でなくても、エンドトキシンの検出を短時間でできる。（検出操作の全工程は従来法：約１．５～２時間に対して、本発明：４０～６０分）

（３）輸血前検査として、本キットを使用することで汚染血液の輸血を未然に防止できる。

（４）従来法では、検体調整から反応停止段階まで、エンドトキシンフリーの操作が必要であるため、使用する試験管ピペットを始め検体以外からのエンドトキシンの汚染を防ぐためクリーンベンチ等清浄な環境下で試験を行う必要があったが、本発明の方法では、測定する血液製剤をディスポシリンジ等で無菌的に採取した後は、クローズドシステムのキット内での反応となるため通常の環境下で行える。

（５）従来の体外診断用医薬品は、赤色を吸光度で読み、正確な定量が可能であるが、本発明の方法は、第１目的を輸血用血液が細菌に汚染されていないかどうかを検出することにおいているため、肉眼により赤色を判断することで判定できるものである。

【図面の簡単な説明】

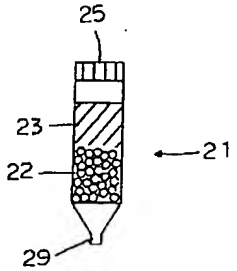
【図１】エンドトキシン検出キットの概略図

【図２】エンドトキシン検出キットの概略図

【符合の説明】

１、２１	エンドトキシン検出キット（検出キット）
２	反応槽
３	反応停止槽
４	着色槽
５、２５	栓体
６、７	接続管
８、９	連通部材
１２	主剤
１３	反応停止剤
１４	着色剤
２２	吸着剤
２３	保存剤
２９	廃液口

【図２】



【図1】